Morphometric Analysis of Craniodental Characters of the House Rat, *Rattus rattus* (Rodentia: Muridae) in Peninsular Malaysia

(Analisis Morfometri Ciri Kraniodental Tikus Rumah *Rattus rattus* (Rodentia: Muridae) di Semenanjung Malaysia)

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ABSTRACT

Rattus rattus *which is believed to be native to India is easily found in all continents of the world. With the absence of* R. rattus *from lineage I and the presence of lineage IV in Southeast Asia, little is known about how rodent morphology varies biogeographically. We evaluate the skull morphometrics in the population of* R. rattus *in Peninsular Malaysia and examine the intraspecific variation of the skull by assessing 20 craniodental characters of 130* R. rattus *specimens. After observing the correlation matrix, highly correlated characters were removed and the remaining 14 craniodental characters were analysed using the conventional morphometrics method via univariate and multivariate statistics. We assessed the impact of age, sex and geographical factors (latitude, longitude and precipitation) on craniodental size. Male and female specimens were analysed separately since statistically significant sexual dimorphism was present. Males showed greater craniodental measurements compared to females. Three distinct age groups (C2, C3 and C4) were visible in the principal component analysis (PCA) and canonical variate analysis (CVA) plots for male and female specimens. Ages C2 and C3 shared similarities in craniodental measurements while C4 showed variability compared to C2 and C3. None of the geographical factors studied showed statistical significance for both male and female* R. rattus. *The results obtained from this study could potentially be a yardstick to observe if similar variations in craniodental traits are present in* R. rattus *from other countries in the Southeast Asian region.*

Keywords: Conventional morphometrics; craniodental; geographic variation; ontogenetic; sexual dimorphism

ABSTRAK

Rattus rattus *yang dipercayai berasal dari India mudah ditemui di setiap benua di dunia. Dengan ketiadaan* R. rattus *daripada 'lineage I' serta kehadiran* R. rattus *daripada 'lineage IV' di Asia Tenggara, sedikit diketahui mengenai bagaimana morfologi tikus berbeza secara biogeografi. Kami menilai morfometri tengkorak populasi* R. rattus *di Semenanjung Malaysia dan mengkaji variasi intraspesies bagi tengkorak dengan menilai 20 ciri-ciri kraniodental bagi 130 spesimen* R. rattus. *Setelah memantau matriks korelasi, ciri-ciri berkorelasi tinggi dikeluarkan dan baki 14 daripada ciri-ciri telah dipilih dan analisis dijalankan berdasarkan kaedah morfometri konvensional menggunakan statistik univariat dan multivariat. Kami mengkaji pengaruh umur, jantina dan faktor-faktor geografi (latitud, longitud dan pemendakan) terhadap saiz kraniodental. Dimorfisme seks yang signifikan secara statistik wujud, maka spesimen jantan dan betina dianalisis secara berasingan. Tikus jantan menunjukkan ukuran kraniodental yang lebih besar berbanding tikus betina. Tiga kumpulan umur yang jelas (C2, C3 dan C4) kelihatan pada plot analisis komponen prinsipal (PCA) dan plot analisis variat kanonikal (CVA) bagi kedua-dua spesimen jantan dan betina. Kumpulan umur C2 dan C3 mempunyai persamaan daripada segi ukuran kraniodental manakala C4 menunjukkan perbezaan yang ketara berbanding C2 dan C3. Kesemua faktor geografi yang dikaji tidak menunjukkan keertiaan berstatistik bagi* R. rattus *jantan dan betina. Hasil yang diperoleh daripada kajian ini berpotensi menjadi panduan untuk dicerap sekiranya variasi yang sama wujud pada ciri-ciri kraniodental dalam* R. rattus *dari negara-negara lain di rantau Asia Tenggara.*

Kata kunci: Dimorfomisme seks; kraniodental; morfometri konvensional; ontogenetik; variasi geografi

INTRODUCTION

The black rat, *Rattus rattus* better recognised as the house rat is extensively circulated in commensal habitats. This rat is medium sized, with relatively large ears and almost a hairless tail that is always longer than the body. This species is typically brown-black to black on the upper parts in colour with a slightly grey to white on the under parts. This species is also the most devastating of all animal pests. Black rats are perceived as hosts and carriers of various zoonotic diseases (leptospirosis, hantavirus and typhus) mainly derived from bacteria, viruses and parasites (Buckle & Smith 2015; Yusof et al. 2010; Zainal Abidin & Noor Azmi 1999). Black rats can easily be found in many habitats mostly in coastal areas, urban areas, farms of temperate region and forested areas. While the rodent distribution is highly dependent on dietary preference,

R. rattus feeds on almost all edible things. This explains why the species is so widespread. The distribution of the *R. rattus* is difficult to pin down and their origins are intensely argued. Nowak (1999) suggested that this species is possibly native to the Malaysian region while Musser and Carleton (2005) specified that these rats are origins of Indian Peninsula. *Rattus rattus* are thought to enter the Malaysian region through human sea faring.

Isthmus of Kra is the narrow neck of Thailand and southern Myanmar linking the Malay Peninsula to the Asian mainland. Biogeographers discovered very significant transitions on the Thai-Malay Peninsula near Isthmus of Kra which is the meeting point of faunas belonging to Indochinese and Sundaic zoogeographic subregions (Woodruff & Turner 2009). A recent work by Aplin et al. (2011) highlighted the absence of *R. rattus* sensu stricto from lineage I of the Indian holotype in Southeast Asia. The specimens found in the north of Isthmus of Kra belonged to lineage IV while those in the southern region were not further described. It is not clearly known how rodent morphology varies biogeographically across Southeast Asia, a region which is comprised of numerous peninsulas/islands. This motivated us to undertake the task of collecting specimens of *R. rattus* in Peninsular Malaysia. Located near the equator, Peninsular Malaysia has a hot and humid climate throughout the year.

Conventional morphometrics which is based on analysis of distances (lengths and widths) has provided the methodological basis for non-geographic and geographic variation study in phenotypic morphological traits. Morphometric methods have been widely applied to investigate skull variation among rodent species (Ben Faleh et al. 2012; Bezerra & De Oliveira 2010; Bohoussou et al. 2014; Lalis et al. 2009; Nicolas et al. 2008), but no studies on craniodental measurements of *R. rattus* have been reported in Peninsular Malaysia.

This study was undertaken to examine the intraspecific variation of the skull characters in *R. rattus* from Peninsular Malaysia using conventional morphometrics. This study also addresses the existence of non-geographic variation (sexual dimorphism and age variation) among *R. rattus* and examines if there is any concurrence of morphometric skull size between populations. To date, no detailed morphometric study on craniodental measurements of *R. rattus* has been performed in Southeast Asia. We aim to provide a baseline for future studies allowing the comparison of skull variation within population of *R. rattus* in Southeast Asia.

MATERIALS AND METHODS

PERMIT APPROVAL AND SPECIMEN COLLECTION

This study was endorsed by the Institutional Animal Care and Use Committee, University of Malaya (UM IACUC) with an ethics reference number ISB/10/06/2016/NHMI (R). The second approval was from The Department of Wildlife and National Parks Peninsular Malaysia where authorised this study in collecting wild specimens with permit reference number; JPHL&TN(IP): 100-34/1.24 Jld. 7(6). This study was carried out without the involvement of any threatened species.

Individuals of *R. rattus* were trapped from 13 cities throughout Peninsular Malaysia. The details of localities and samples sizes (*N*) from which *R. rattus* specimens were collected in Peninsular Malaysia are shown in Figure 1 and Table 1. Trapping was conducted over a period of four days and three nights for each session at every sampling site with a total of 30 trapping sessions organised between December 2016 and March 2019. Thirty cage traps sized 28 cm × 15 cm × 12 cm baited with banana, coconut or bread coated with peanut butter were placed at each site. The traps were set up in the late evening and checked early morning. These specimens were collected with the help from municipalities of each city as part of their vector control programme. Sampling was carried out in various sites (i.e. seaside, housing areas and fresh markets) with emphasis on populated areas as the study organisms are usually found near human habitation.

All live rats were identified based on their external size and features by referring to the taxonomic keys (Aplin et al. 2003; Corbet & Hill 1992; Francis 2008; Wilson & Reeder 2005). All specimens were euthanised in a sealed container connected to a carbon dioxide (CO_2) tank. Dead specimens then underwent dissection procedures after information such as photos, external measurements, sex and weight were recorded. All skulls were extracted for the morphometrics study.

CONVENTIONAL MORPHOMETRICS

The cranial and mandible characters of *R. rattus* were measured using the digital vernier caliper (Mitutoyo Co., Kanagawa, Japan) and values were rounded to the nearest 0.01 mm. We measured 20 morphometric variables from the *R. rattus* skulls as shown in Figure 2 based on four different parts of measurements referring to Musser and
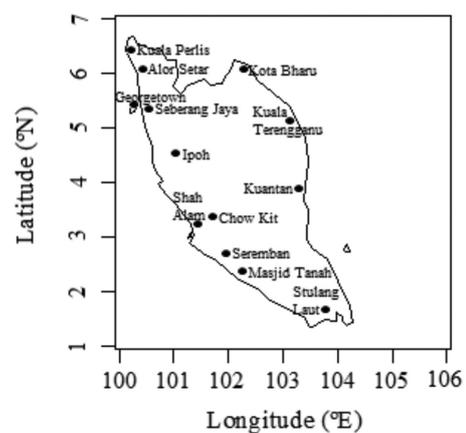
FIGURE 1. A map illustrating the sampling locations of *R. rattus* throughout Peninsular Malaysia

TABLE 1. The localities and samples sizes *(N)* from which *R. rattus* populations were collected in Peninsular Malaysia

Locality	Latitude (North)	Longitude (East)	Mean of precipitation (mm year⁻¹)	N	Habitat
Kuala Perlis, Perlis	6° 23' 52.4"	100° 7' 50.4"	2133	9	Seaside
Kota Bharu, Kelantan	6° 8' 23.54"	102° 14' 31.93"	2558	8	Housing area
Alor Setar, Kedah	6° 7' 29.3"	100° 22' 4.1"	2276	12	Housing area
Georgetown, Penang island	5° 26' 8.3"	100° 18' 32.8"	2462	6	Seaside
Seberang Jaya, Penang mainland	5° 24' 4.5"	100° 23' 51.1"	2425	7	Housing area
Kuala Terengganu, Terengganu	5° 19' 48.72"	103° 8' 26.88"	2736	10	Housing area
Ipoh, Perak	4° 35' 50.9"	101° 5' 24.4"	2435	8	Fresh market
Kuantan, Pahang	3° 48' 27.7"	103° 19' 33.6"	2887	12	Housing area
Chow Kit, Kuala Lumpur	3° 9' 35.9"	101° 41' 49"	2486	15	Fresh market
Shah Alam, Selangor	3° 4' 23.8"	101° 31' 6.5"	2304	9	Housing area
Seremban, Negeri Sembilan	2° 43' 33.2"	101° 56' 16.2"	2043	12	Fresh market
Masjid Tanah, Melaka	2° 21' 8.2"	102° 6' 32.1"	2101	10	Housing area
Stulang Laut, Johor	1° 28' 10.3"	103° 46' 53.9"	2554	12	Seaside
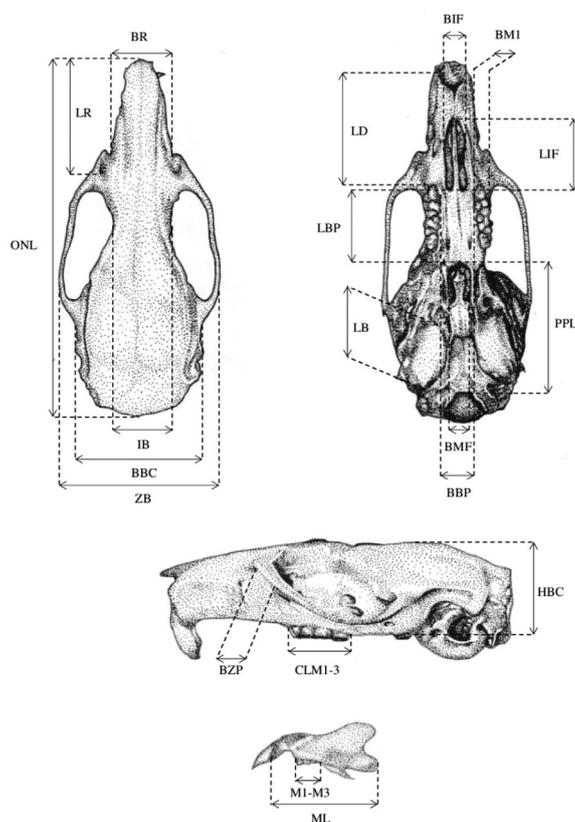
FIGURE 2. Craniodental measurements of *R. rattus* based on dorsal,
ventral and lateral views used in morphometric analyses

Newcomb (1983) and Musser et al. (2006) which consist of: dorsal - occipitonasal length, or the greatest length of the skull (ONL), length of rostrum (LR), breadth of rostrum (BR), zygomatic breadth (ZB), breadth of braincase (BBC), interorbital breadth (IB); ventral - length of diastema (LD), length of incisive foramina (LIF), breadth of incisive foramina (BIF), breadth of first upper molar (BM1), length of bony plate (LBP), length of auditory bulla (LB), postpalatal length (PPL), breadth of mesopterygoid fossa (BMF), breadth across palate at first molars (BBP); lateral - breadth of zygomatic plate (BZP), crown length of maxillary molar row (CLM1-3), height of braincase (HBC); and mandible - length of mandible (ML), length of mandible toothrow (M1-M3).

The exact biological age of the specimens could not be identified in this study since these rats were wild-

caught during trapping sessions. Hence, age classes were described on the basis of molar wear stages following Ben Faleh et al. (2013) (Figure 3) and interpreted as: Stage C0: No upper M3 is erupted; Stage C1: Upper M3 erupted but not worn; Stage C2: Cusps still visible on all molars and link between first and second lobe of the upper M3 is very narrow; Stage C3: The longitudinal link between first and second lobe of upper M3 is larger and generally wider; Stage C4: The upper M3 displays nearly the total fusion of the first and second lobe of the longitudinal link that is very wide but it remains visible on the other molars cusps; Stage C5: No more cusps were visible and the longitudinal link is so wide that lobes appeared fused on all the molars.
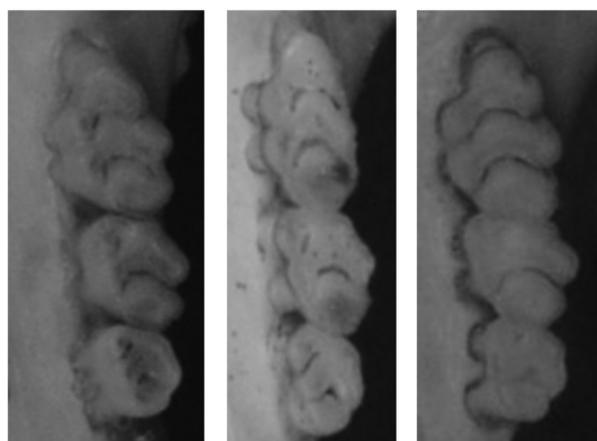
FIGURE 3. Different age classes of *R. rattus* (left to right) proportional to C2, C3 and C4, respectively

TABLE 2. t-test for difference in mean of craniodental measurements between male and female *R. rattus*

Craniodental measurements	P
ONL	***
BBC	***
LD	*
LIF	***
BM1	***
LBP	*
LB	***
BMF	**
BBP	**
BZP	**
CLM1-3	***
HBC	***
ML	0.3416 (NS)
M1-M3	***

*** $P < 0.0001$, ** $P < 0.01$, * $P < 0.05$, NS - not significant

STATISTICAL ANALYSIS

Statistical analyses were executed using Morpho (Schlager 2017), maps (Richard et al. 2018), MASS (Venables & Ripley 2002) and mapdata (Kahle & Wickham 2013)

packages in R version 3.5.1 (R Core Team 2015). We measured 20 morphometric variables from the *R. rattus* skulls. To avoid multicollinearity, we computed the correlation matrix and removed highly correlated variables (correlation coefficient above 0.85) such as LR, IB, BIF, PPL, ZB and BR and the morphometric variables examined reduced to 14 variables.

We conducted the analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA) to check the impact of sexual dimorphism and age on the size of the skull. Regressions were performed on the log of skull size versus geographical factors such as latitude, longitude and precipitation for males and females separately since sexual dimorphism was present.

We investigated the significance of differences between males and females for every craniodental measurement. The 'a posteriori' Scheffe's test was performed as a post hoc procedure to study the significance of difference in ages for each of the 14 morphometric variables. General trends of the skull sizes were studied via principal component analysis (PCA) for the 14 standardised craniodental measurements of males and females separately due to the presence of sexual dimorphism. In addition, we assessed the morphometric differences of the specimens via canonical variate analysis (CVA) where specimens were considered as clades.

RESULTS

A total of 130 individuals of *R. rattus* were caught and examined for skull morphometrics study. The skulls measured comparing individuals from age groups varying from C2 to C4, were employed in the statistical analysis. Craniodental measurements of *R. rattus* were significantly associated with age ($P < 0.0001$) and sex ($P < 0.0001$), and showed the presence of sexual dimorphism. The sex ratio was rather equilibrated with 67 males and 63 females. Based on the regression analysis, all three geographical factors studied (i.e. latitude, longitude and precipitation) were not statistically significant for male and female. All variables of craniodental measurements showed significant difference between males and females except ML (Table 2), therefore, further analyses were performed separately. Generally, males portrayed higher craniodental measurements compared to females.

The PCA plots for males and females (Figure 4) showed three groups respectively; specimens of age C2: males ($N = 15$), females ($N = 24$); specimens of age C3: males ($N = 16$), females ($N = 14$); and specimens of age C4: males ($N = 36$), females ($N = 25$). These plots showed that age groups C2 and C3 shared similar measurements and significant difference can be seen between these groups with age C4.

For males, axes 1 and 2 of the PCA plot explained 86.6% of the total variation where 82.8% of the total variation was described by axis 1 while 3.8% was described by axis 2. Based on the results in Table 3, PC1 was negatively correlated with all the variables, PC2 showed strongest negative correlation with HBC and PC3 showed
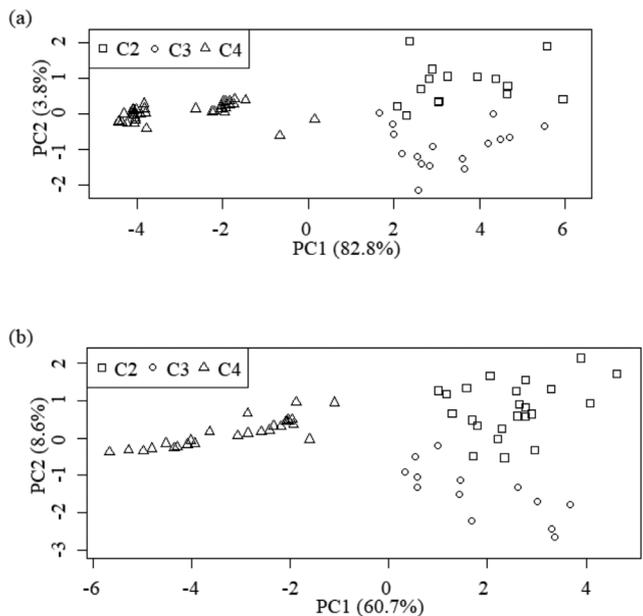
FIGURE 4. PC1 and PC2 of the PCA for 14 craniodental measurements
in (a) 67 male and (b) 63 female *R. rattus*

strongest positive correlation with ML and M1-M3. The canonical variate analysis based on the PCA results showed three age groups (Figure 5) with C2 and C3 sharing similar characteristics while C4 was significantly different. The plot of the canonical variates, CV1 and CV2 displayed separations between the specimens from age groups C2, C3 and C4. CV1 displayed 77.6% of the total variation while CV2 accounted 22.4%.

For females, the first two axes of the PCA plot accounted to 69.3% of the total variation where axis 1 and axis 2 explained 60.7% and 8.6% of the total variations respectively. From Table 3, PC1 displayed negative correlations with all the variables, PC2 and PC3 showed the highest negative correlation with HBC. The highest positive correlations involving PC2 were seen with LD and LBP. The CVA plot for the female *R. rattus* also showed three age
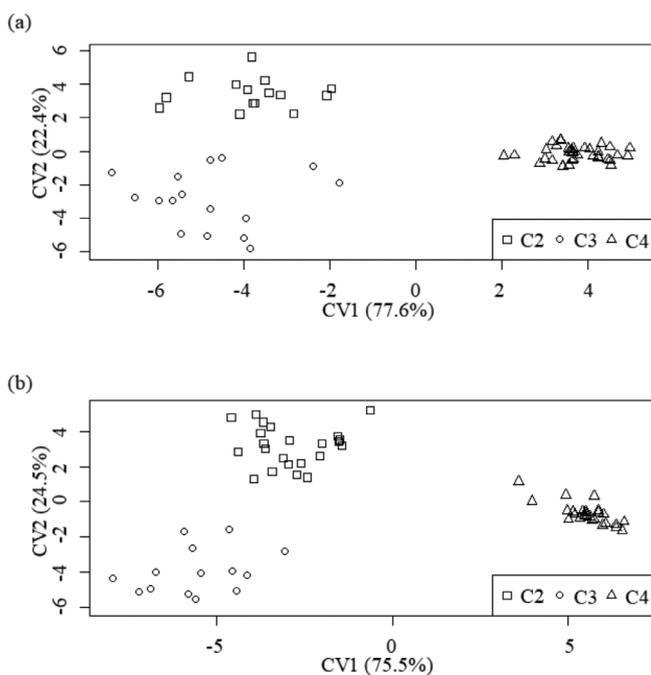
FIGURE 5. Results of CVA of the 14 craniodental measurements examined
for (a) male and (b) female *R. rattus*

groups by displaying three clusters (Figure 5). Axis CV1 accounted to 75.5% of the total variation and CV2, 24.5%.

The 'a posteriori' Scheffe's test comparing 14 craniodental measurements individually between age groups for males and females (Table 4) showed that the age groups C2 and C3 have some craniodental measurements which do not differ significantly. However, the differences became more prominent when C2 and C3 were compared with C4 for both genders.

DISCUSSION

This study showed the craniodental variability in *R. rattus* across Peninsular Malaysia. Present study attempted to deliver some knowledge for genetically delimitated species based on assessment of morphological data. Our approach enabled us for the first time to elucidate some of the sizes of skull variability in *R. rattus* populations in Peninsular Malaysia. The utilisation of conventional morphometric techniques in evaluating sexual dimorphism and ontogenetic variation in the present study demonstrated statistically significant variations among craniodental sizes of *R. rattus*. Generally, it is known that male *R. rattus* tend to be larger in size compared to the female counterparts. Sexual dimorphism found in present study was similar to previous studies (Abdel-Rahman et al. 2008; Martínez et al. 2014). However, absence of sexual dimorphism was seen in western Mediterranean Moroccan and Tunisian black rat populations (Ben Faleh et al. 2013; Ventura & Lopez-Fuster 2000). Distinctive growth trends within the genders may prompt to diverse phenotypic patterns during the ontogeny of *R. rattus*, suggesting that sexual dimorphism in craniodental size could be due to natural selection influencing separately in the two genders during growth

development inducing in opposite outcomes (Bronson 1989). Furthermore, behaviours may also influence the sexual dimorphism in rats (Bronson 1989). The reason for males to be larger in size is yet to be known due to the lack of common ecological inspections on *R. rattus* in Peninsular Malaysia.

Geographical factors such as latitude, longitude and precipitation do not affect the craniodental measurements of *R. rattus* in the region studied because it is located in the equatorial region and does not vary significantly in longitude. Aplin et al. (2011) presented the genetic examination of the settlement of *R. rattus* in mainland Asia using mitochondrial sequences. Four to six different species of the *R. rattus* complex (RrC), with six lineages (I–VI) were identified with *R. rattus* lineage I dispersed in India, Africa, America, Europe and Australia while lineage IV covered remote areas in East Asia including the Indochinese Peninsula (Aplin et al. 2011) via Indonesia and Philippines. However, lineage IV was not further distributed towards the south to Australia and Papua New Guinea where *R. rattus* lineage I was detected. Phylogenetic investigation of *R. rattus* by Aplin et al. (2011) specified that these two lineages were principally allopatric and developing in isolation. The phylogenetic revision has involved specimens of black rats from numerous geographical regions in Asia but with the absence of specimens from Peninsular Malaysia. However, they postulated that the lineage that was hypothesised to be distributed across Peninsular Malaysia (*R. rattus cf.* lineage IV sensu Aplin et al. 2011) is not the *Rattus rattus* sensu stricto, apparently native of India.

The principal components are created such that the PC1 accounts for the maximum variation, PC2 for as much as the remaining and so forth. Based on the PCA plots, the

TABLE 3. Variable coefficients and percentage variation described by the first three components (PC1-PC3) of the PCA on 14 standardised craniodental measurements from male and female *R. rattus* specimens

Craniodental measurements	Male			Female		
	PC1	PC2	PC3	PC1	PC2	PC3
ONL	-0.2805	0.0598	-0.0272	**-0.3102***	0.0882	-0.0732
BBC	-0.2723	0.0375	0.3056	-0.2967	-0.2690	0.2575
LD	-0.2782	0.1496	0.0318	-0.2667	**0.4374***	-0.3115
LIF	-0.2780	0.0991	-0.1744	-0.2318	0.0537	0.0868
BM1	-0.2696	0.1376	-0.1053	-0.2745	0.0561	0.2412
LBP	-0.2656	0.3015	0.3229	-0.2473	**0.4419***	-0.207
LB	-0.2671	-0.0173	-0.0114	-0.2541	-0.0425	**0.4486***
BMF	-0.2698	0.2625	-0.1273	-0.2923	0.2145	-0.2832
BBP	-0.2807	0.0199	-0.0990	-0.2984	-0.0493	-0.0866
BZP	-0.2687	-0.0499	-0.1300	-0.2911	-0.3404	-0.1421
CLM1-3	-0.2781	0.1345	-0.0841	-0.2883	0.0239	0.2728
HBC	-0.2230	**-0.7816***	-0.3453	-0.1567	**-0.5685***	**-0.5219***
ML	-0.2541	-0.2033	**0.5692***	-0.2658	-0.0562	0.2289
M1-M3	-0.2501	-0.3325	**0.5155***	-0.2288	-0.1847	-0.1198
% variance	82.81	3.84	2.98	60.73	8.61	7.08

*Asterisk in the table denote variables that explain the highest percentage of variation on each principal component (PC)

TABLE 4. Results of 'a posteriori' Scheffe's test to assess the significant differences of craniodental measurements between ages C2, C3 and C4 for male and female *R. rattus* specimens

Craniodental measurements	Male			Female		
	C2 and C3	C2 and C4	C3 and C4	C2 and C3	C2 and C4	C3 and C4
ONL	0.9804 (NS)	***	***	0.9425 (NS)	***	***
BBC	0.8427 (NS)	***	***	***	***	***
LD	0.2004 (NS)	***	***	***	***	***
LIF	0.8425 (NS)	***	***	0.9981 (NS)	***	***
BM1	0.9949 (NS)	***	***	0.8773 (NS)	***	***
LBP	**	***	***	***	***	***
LB	0.6695 (NS)	***	***	"	***	*
BMF	0.1438 (NS)	***	***	**	***	***
BBP	0.9721 (NS)	***	***	"	***	***
BZP	*	***	***	***	***	***
CLM1-3	0.8863 (NS)	***	***	0.1442 (NS)	***	***
HBC	***	***	**	**	***	0.7864 (NS)
ML	0.5627 (NS)	***	***	"	***	***
M1-M3	0.3240 (NS)	***	***	0.7546 (NS)	***	**

*** $P < 0.0001$, ** $P < 0.01$, * $P < 0.05$, " $P < 0.1$, NS - not significant

PC1 scores were fairly negative indicating that the first principal component score increases with the decrease in all the 14 variables studied. This implies that the high PC1 score is associated with smaller measurements of all the 14 measurements studied. The eigenvalues for PC2 and PC3 were much smaller compared to those of PC1 showing that PC1 accounts for majority of the craniodental measurements considered. The highest negative correlation in HBC with PC2 reflects that the size of HBC decreases as PC2 increases and the decrease in HBC has the greatest influence in the variability of PC2. For PC3, the increase in the size of ML and M1-M3 is responsible for the variation in PC3. The similar interpretation is applicable for the case of the female *R. rattus* involving HBC for the variation of PC2 and PC3 (highest negative correlation) as well as LD and LBP for the variation of PC2 (highest positive correlation). Variability was evident with the variation in craniodental size among three clades related to age. The variation between these groups was associated to age even though the existing specimens did not represent skulls with age classes belonging to stages C0, C1 and C5. It is difficult to trap individuals of ages C0 and C1 as individuals of these age groups are at infant stages, have restricted movements and are still dependent on their mothers. On the other hand, individuals of age C5 belong to the very elderly group where most tend to be sickly and die. Three age groups i.e. C2, C3 and C4 related to the three clades are evident in the PCA and CVA plots. Every tooth-wear class is morphologically different in size and shape (Figure 3). Within *R. rattus*, individuals of tooth-wear classes 2 and 3 are presumably sub-adults while those with tooth-wear class 4 represent adults. *R. rattus* belonging to age groups C2 and C3 shared some similarity in craniodental measurements and it was anticipated that there would not be much difference in terms of size between these age groups. The significant difference could be seen clearly in age group C4 because this was the adult group. The dentition of *R. rattus* is monophyodont where they only possess one generation of teeth throughout their entire life. Moreover, different age groups have distinctive fundamental growth attributes.

CONCLUSION

Our analyses showed the presence of sexual dimorphism among *R. rattus* in Peninsular Malaysia. The existence of three clusters which represent the age groups was seen in the male and female specimens with ages C2 and C3 sharing some similarities and C4 portraying extensive variability compared to the aforesaid age groups. Geographical factors do not affect the craniodental sizes of *R. rattus* in Peninsular Malaysia. This present study will provide a framework to researchers interested in investigating morphological variation of *R. rattus* in Southeast Asia since no detailed morphometric study has been done to date. Nonetheless, this study may be valuable to show some significant taxonomical queries and lead to proper taxonomic names usage, from the basic background of morphological variability revealed by populations of *R. rattus* found in Peninsular Malaysia. Further genetic studies will be worth to verify the lineage classification of *R. rattus* in Peninsular Malaysia.

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